FORM PTO-1390 (Modified) (REV 11-2000) U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **216120US0PCT**

U S APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/926566**

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/03135	**16 May 2000**	**19 May 1999**

TITLE OF INVENTION
PROLYLENDOPEPTIDASE INHIBITORS

APPLICANT(S) FOR DO/EO/US
Hiroshi KAYAHARA, et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Drawings (2 Sheets)
PCT/IB/308
Notice of Priority
Request for Consideration of Documents Cited in the International Search Report

U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/926566	PCT/JP00/03135	216120US0PCT

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**	
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	

ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	15 - 20 =	0	x $18.00	**$0.00**	
Independent claims	6 - 3 =	3	x $84.00	**$252.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,272.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,272.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,272.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$1,272.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$1,272.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Surinder Sachar
Registration No. 34,423

22850

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

Nov. 19 2001
DATE

#4/a

216120US-0-PCT

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF :

HIROSHI KAYAHARA ET AL : ATTN: APPLICATION DIVISION

SERIAL NO: 09/926,566 :

FILED: NOVEMBER 19, 2001 :

FOR: PROLYLENDOPEPTIDASE INHIBITORS :

PRELIMINARY AMENDMENT

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Responsive to the Official Correspondence dated December 13, 2001, Applicants submit herewith an Amendment, a Sequence Listing, and a corresponding computer-readable Sequence Listing. Prior to examination on the merits, please amend the above-identified application as follows.

IN THE SPECIFICATION

Please amend the specification as follows:

Please replace the paragraph at page 14, lines 9-10, as follows:

--Fig. 1 illustrates the cleavage sites in cerebral functional peptides (SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, and SEQ ID NO. 4) by prolylendopeptidase.--

Page 27 (Abstract), after the last line, beginning on the next page, please delete the Sequence Listing and insert the Sequence Listing attached hereto.

IN THE CLAIMS

Please amend the claims as follows:

--1. (Amended) A prolylendopeptidase-inhibitive agent, comprising an extract of a cereal grain as an active component.

2. (Amended) The prolylendopeptidase-inhibitive agent according to Claim 1, wherein the cereal grain is a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame.

3. (Amended) The prolylendopeptidase-inhibitive agent according to Claim 2, wherein the grain of rice plant is in a germinated form.

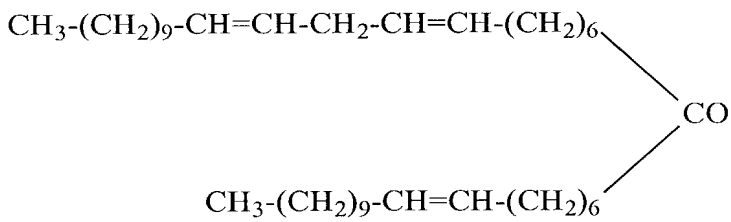
4. (Amended) A method for preparing a prolylendopeptidase-inhibitive agent comprising extracting a cereal grain with water, organic solvent, or mixtures thereof.

5. (Amended) The method according to Claim 4, wherein the cereal grain is a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame.

6. (Amended) The method according to Claim 5, wherein the grain of rice plant is in a germinated form.

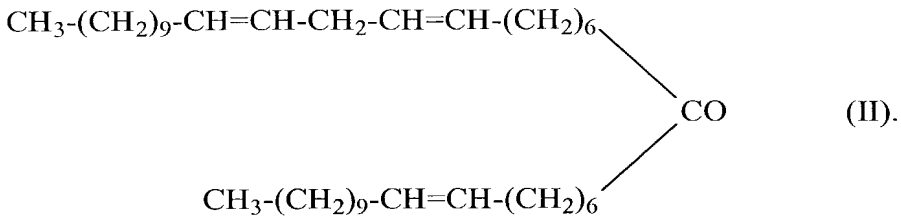
7. (Amended) The method according to Claim 4, wherein the organic solvent is hexane.

8. (Amended) A compound of formula (II)



(II).

9. (Amended) A method for preparing a compound of formula (II),



(II).

comprising extracting a cereal grain and then isolating the compound from the extract.

10. (Amended) The method according to Claim 9, wherein the cereal grain is a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame.

11. (Amended) The method according to Claim 10, wherein the grain of rice plant is in the form of germinated brown rice.

12. (Amended) A prolylendopeptidase-inhibitive agent, comprising the compound according to Claim 8 as an active component.

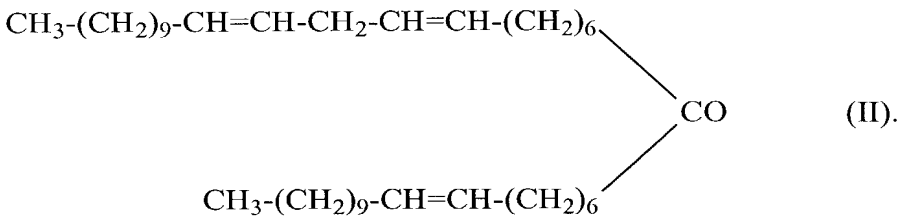
13. (Amended) A food product, comprising a compound according to Claim 8 as an active component.

14. (Amended) A germinated brown rice, comprising prolylendopeptidase-inhibition activity.

15. (Amended) A food product, comprising the germinated brown rice according to Claim 14.--

Please add the following Claims.

--16. (New) The germinated brown rice according to Claim 14, comprising a compound of formula (II)



(II).

17. (New) A method of preventing, reducing, treating, arresting, or alleviating cerebral dysfunction in a human or animal, comprising

administering the food product according to Claim 13 to a human or animal in which said preventing, reducing, treating, arresting, or alleviating is intended.

18. (New) The method according to Claim 17, wherein the cerebral dysfunction is Alzheimer's disease.

19. (New) A method of preventing, reducing, treating, arresting, or alleviating the symptoms of cerebral dysfunction in a human or animal, comprising

administering the food product according to Claim 13 to a human or animal in which said preventing, reducing, treating, arresting, or alleviating is intended.

20. (New) The method according to Claim 17, wherein the cerebral dysfunction is Alzheimer's disease.--

REMARKS

Claims 1-20 are active in the present application.

Claims 1-15 are amended to comply with proper claim form. Claims 16-20 are added. The amendment to the claims and the additional claims are supported at pages 23 and the original claims. No new matter is believed to be introduced by the amendment and the addition of new claims.

Applicants have now submitted a Sequence Listing and a corresponding computer-readable Sequence Listing. Contents of the paper copy of the Sequence Listing and the computer-readable Sequence Listing are identical. Support for all the sequences listed in the Sequence Listing can be found in the present application. No new matter is introduced by the submission of the Sequence Listing and the computer-readable Sequence Listing.

Applicants submit that this application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,
MAIER & NEUSTADT, P.C.

Norman F. Oblon
Attorney of Record
Registration No. 24,618

James J. Kelly, Ph.D.
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Marked-Up Copy
Serial No: 09/926,566
Amendment Filed on:
HEREWITH

IN THE SPECIFICATION

Please amend the specification as follows:

Please replace the paragraph at page 14, lines 9-10, as follows:

--Fig. 1 illustrates the cleavage sites in cerebral functional peptides (SEQ ID NO. 1, SEQ ID NO. 2, SEQ ID NO. 3, and SEQ ID NO. 4) by prolylendopeptidase.--

IN THE CLAIMS

Please amend the claims as follows:

--1. (Amended) A prolylendopeptidase-inhibitive [agent] agent, comprising an extract of a cereal grain as an active component.

2. (Amended) The prolylendopeptidase-inhibitive agent according to [claim] Claim 1, wherein the cereal grain is a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame.

3. (Amended) The prolylendopeptidase-inhibitive agent according to [claim] Claim 2, wherein the grain of rice plant is in a germinated form.

4. (Amended) A method for preparing a prolylendopeptidase-inhibitive agent comprising extracting a cereal grain with [water and/or an] water, organic [solvent] solvent, or mixtures thereof.

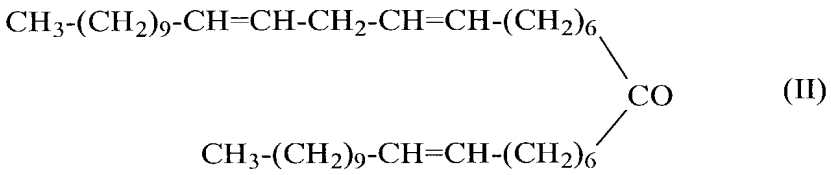
5. (Amended) [A] The method according to [claim] Claim 4, wherein the cereal grain is a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame.

6. (Amended) The method according to [claim] Claim 5, wherein the grain of rice plant is in a germinated form.

7. (Amended) The method according to [claim] Claim 4, wherein the organic solvent is hexane.

8. (Amended) A compound of formula[(II).] (II)

[



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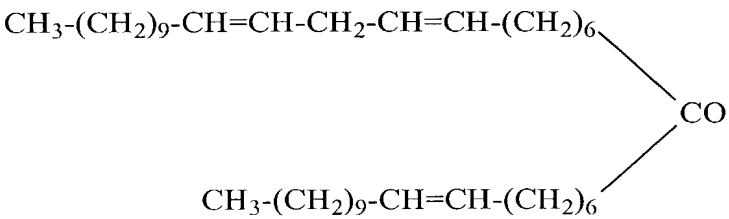
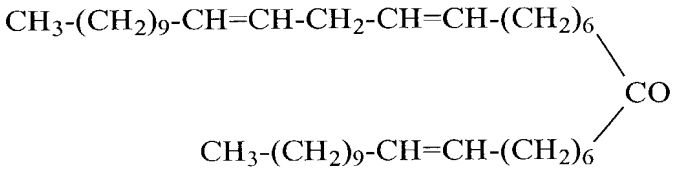
]



(II).

9. (Amended) A method for preparing a compound of formula (II), [comprising extracting a cereal grain and then isolating the compound from the extract.]



(II)

comprising extracting a cereal grain and then isolating the compound from the extract.

10. (Amended) The method according to [claim] Claim 9, wherein the cereal grain is a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame.

11. (Amended) The method according to [claim] Claim 10, wherein the grain of rice plant is in the form of germinated brown rice.

12. (Amended) A prolylendopeptidase-inhibitive [agent] agent, comprising [a] the compound [as claimed in claim] according to Claim 8 as an active component.

13. (Amended) A food [product] product, [for preventing and/or improving cerebral dysfunctions, the food product] comprising a compound [as claimed in claim] according to Claim 8 as an active component.

14. (Amended) A germinated brown [rice] rice, [for preventing and/or improving cerebral dysfunctions, the germinated brown rice having a] comprising prolylendopeptidase-inhibition activity.

15. (Amended) A food [product] product, [for preventing and/or improving cerebral dysfunctions, the food product] comprising [a] the germinated brown rice [having a prolylendopeptidase-inhibition activity] according to Claim 14.--

--Claims 16-20 is new.--

Rec'd PCT/PTO 13 MAR 2002

Docket No. 216120US0PCT

IN RE APPLICATION OF: Hiroshi KAYAHARA, et al.

SERIAL NO: 09/926,566

FILED: November 19, 2001

FOR: PROLYLENDOPEPTIDASE INHIBITORS

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

SIR:

Transmitted herewith is an Preliminary Amendment w/Marked-Up Copy (9 pp.) in the above-identified application.

- ☐ No additional fee is required
- ☐ Small entity status of this application under 37 C.F.R. §1.9 and §1.27 is claimed.
- ■ Additional documents filed herewith: Substitute Sequence Listing Paper (2 pp.); Substitute Sequence Listing Computer Readable Form (CRF) Diskette; Declaration, Power of Attorney and Petition (4 pages executed); Filing of Declaration Under 37 CFR 1.53(f); Return Copy - Notification of Missing Requirements under 35 USC 371 in the U.S. Designated/Elected Office (DO/EO/US); Request for Extension of Time (one month).

The Fee has been calculated as shown below:

CLAIMS	CLAIMS REMAINING		HIGHEST NUMBER PREVIOUSLY PAID	NO. EXTRA CLAIMS	RATE	CALCULATIONS
TOTAL	20	MINUS	20	0	× $18 =	$0.00
INDEPENDENT	6	MINUS	6	0	× $84 =	$0.00
		☐ MULTIPLE DEPENDENT CLAIMS			+ $280 =	$0.00
		TOTAL OF ABOVE CALCULATIONS				$0.00
		☐ Reduction by 50% for filing by Small Entity				$0.00
		☐ Recordation of Assignment			+ $40 =	$0.00
					TOTAL	$0.00

- ■ A check in the amount of **$110.00** is attached.
- ■ Please charge any additional Fees for the papers being filed herewith and for which no check is enclosed herewith, or credit any overpayment to deposit Account No. 15-0030. A duplicate copy of this sheet is enclosed.
- ■ If these papers are not considered timely filed by the Patent and Trademark Office, then a petition is hereby made under 37 C.F.R. §1.136, and any additional fees required under 37 C.F.R. §1.136 for any necessary extension of time may be charged to Deposit Account No. 15-0030. A duplicate copy of this sheet is enclosed.

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(OSMMN 10/00)

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Norman F. Oblon
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James J. Kelly, Ph.D.
Registration No. 41,504

SEQUENCE LISTING

<110> KAYAHARA, HIROSHI
TSUKAHARA, KIKUICHI
INAGAKI, TAKESHI

<120> L-AMINO ACID OXIDASE FROM RHODOCOCCUS SPECIES

<130> 216120US0PCT

<140> US 09/926.566
<141> 2001-11-19

<150> JP 11-138791
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DESCRIPTION

PROLYLENDOPEPTIDASE INHIBITORS

FIELD OF THE INVENTION

The present invention relates to a prolylendopeptidase-inhibitor comprising an extract of a cereal grain as an active component; a method for preparing the prolylendopeptidase-inhibitor; a compound having a prolylendopeptidase-inhibition activity, which is extracted and isolated from a cereal grain; a method for preparing the compound; a prolylendopeptidase-inhibitor comprising the compound; a food product for preventing and/or improving cerebral dysfunctions, which comprises the compound; a germinated brown rice for preventing and/or improving cerebral dysfunctions, which has a prolylendopeptidase-inhibition activity; and a food product for preventing and/or improving cerebral dysfunctions, which comprises the germinated brown rice.

BACKGROUND ART

With the oncoming of the aging society, senile dementia has becomes a serious social problem. Senile dementia has been mainly classified into two types: a neuron-disorder type, which is caused by the disorder of brain neurons; and a non-neuron-disorder type, which is caused by the disorder of brain tissues other than brain neurons (e.g., vascular thrombosis). One of the neuron-disorder type of dementias is Alzheimer's disease (AD).

In patients suffering from AD, the brain is affected progressively, and some symptoms are developed such as poriomania, incontinence, illusion, paranoia, loss of memory and personality

disintegration, eventually resulting in death within 2-15 years after the onset even though there is a variation among individuals. Since AD patients are less declined in their motile ability, they may behave a frequent wandering off and so on, which becomes too burdensome for those who provide daily care for them.

The cause of AD is not yet completely clear, but some pathologic manifestations have been recognized. For example, in addition to the remarkable atrophy of the brain, the following manifestations are observed in the brain of AD patients as the hallmark of the disease: (1) quantitative abnormality of physiologically active substances in the brain; (2) senile plaques composed primarily of β-amyloid peptides accumulated outside the nerve cells; and (3) distortion of neurofibrils composed primarily of highly phosphorylated tau protein accumulated within the neuron cells. The manifestation of item (1) is assumed to be caused by stimulation of the degradation of cerebral function-associated peptides with prolylendopeptidase (PEP). PEP is a serine protease which has a specificity for proline residue present in a peptide chain and cleaves the peptide chain at the carboxyl side of the proline residue as illustrated in Fig. 1. It is presumed that PEP cleaves a proline-containing cerebral function-associated peptide which permits the brain to function normally (e.g., substance P and neurotensin as neurotransmitters; and vasopressin and oxitocin as peptides involved in the memory function) to inactivate the peptide, thereby causing decrease in the amount of the peptide in the brain and derangement of the cerebral function and ultimately causing the development of AD. In fact, it has been evidenced that the amount of vasopressin in a patient with dementia is lower than that in a

normal person (BIOINDUSTRY 4:788-796, 1987).

It is therefore expected that substances capable of specifically inhibiting PEP might be effective for prevention and treatment of various PEP-associated disorders (e.g., amnesia), as well as AD. As such substances, there have been reported synthetic PEP-inhibitors such as N-acyl pyrrolidine derivatives (Japanese Patent Application Laid-open Nos. 61-37764, 61-183297 and 61-238775) and pyrrolidine amide derivatives (Japanese Patent Publication No. 7-64834), and PEP-inhibitive peptides derived from *sake* lees (Japanese Patent Application Laid-open No. 10-77300). However, from the viewpoints of safety and so on, PEP-inhibitive substances derived from natural materials are demanded.

With the increased tendency of chronic diseases and the oncoming of the aging society, there is a growing consciousness about the prevention of diseases for adults and elderly people through the uptake of daily foods. Up to now, several food products for patients having specific diseases such as hypertension and constipation have been commercialized. However, no food products for patients having senile dementia and amnesia have been yet known.

DISCLOSURE OF THE INVENTION

The object of the present invention are to provide a prolylendopeptidase-inhibitive agent comprising an extract of a cereal grain as an active component; a method for preparing the prolylendopeptidase-inhibitive agent; a compound having a prolylendopeptidase-inhibition activity, which is extracted and isolated from a cereal grain; a method for preparing the compound;

a prolylendopeptidase-inhibitive agent comprising the compound; a food product for preventing and/or improving cerebral dysfunctions, which comprises the compound; a germinated brown rice for preventing and/or improving cerebral dysfunctions, which has a prolylendopeptidase-inhibition activity; and a food product for preventing and/or improving cerebral dysfunctions, which comprises the germinated brown rice.

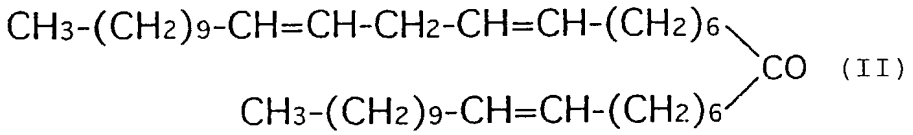
As a result of intensive and extensive researches for overcoming the problems set forth above, the present inventors have found a substance capable of specifically inhibiting PEP in a rice grain, and have succeeded in isolation and purification of the substance from a germinated brown rice. The finding and success lead to the accomplishment of the invention.

It is therefore an object of the present invention to provide a prolylendopeptidase-inhibitive agent comprising an extract of a cereal grain as an active component. The cereal grain may be a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame. The grain of rice plant may be in a germinated form (e.g., a germinated brown rice).

It is another object of the present invention to provide a method for preparing a prolylendopeptidase-inhibitive agent comprising extracting a cereal grain with water and/or an organic solvent(e.g. hexane). The cereal grain may be a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame. The grain of rice plant may be in a

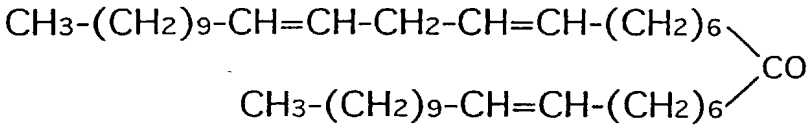
germinated form (e.g., a germinated brown rice).

It is still another object of the present invention to provide a compound of formula (II).



(II)

It is still another object of the present invention to provide a method for preparing a compound of formula (II), comprising extracting a cereal grain and then isolating the compound from the extract.



(II)

The cereal grain may be a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame. The grain of rice plant may be in a germinated form (e.g., a germination brown rice).

It is still another object of the present invention to provide a prolylendopeptidase-inhibitive agent comprising a compound as described above as an active component.

It is still another object of the present invention to provide a food product for preventing and/or improving cerebral dysfunctions, the food product comprising a compound as described above as an active component.

It is still another object of the present invention to provide a germinated brown rice for preventing and/or improving cerebral dysfunctions, the germinated brown rice having a

prolylendopeptidase-inhibition activity.

It is still another object of the present invention to provide a food product for preventing and/or improving cerebral dysfunctions, the food product comprising a germinated brown rice having a prolylendopeptidase-inhibition activity.

This specification includes part or all of the contents as disclosed in the specification and/or drawings of Japanese Application No.11-138791, which is a priority document of the present application.

Hereinbelow, the present invention will be illustrated in detail.

The PEP-inhibitive compound according to the present invention is a specific ketone or glyceride derived from cereal grains, which is distinctive from the conventional PEP-inhibitors. The PEP-inhibitive compound can be isolated and purified in the following manner.

1. PEP-inhibitive Compound of the Invention

(1) Source of PEP-inhibitive compound

The source of the PEP-inhibitive compound of the present invention may be a grain or seed of a cereal crop, such as rice plant, wheat, corn, soybean, mile, buckwheat, foxtail millet, barnyard grass, proso or sesame, preferably a brown rice grain, more preferably a germinated brown rice grain. The source may also be a rice-derived material such as rice bran, rice germ and rice-bran oil. Examples of the brand names of the rice include, but are not limited to, *Koshihikari*, *Akitakomachi* and *Chugoku* #137.

The germinated brown rice can be prepared by immersing brown

rice in water or warmed water adjusted to a temperature from 5 to 50°C, preferably from 30 to 34°C for a given time period. Specifically, brown rice grains are immersed in warmed water until the grains absorb water sufficiently, and then removed out therefrom. The water-absorbed brown rice grains are left to stand in a highly humid atmosphere (e.g., relative humidity of 100%) for a time period from 5 hours to 5 days, preferably from 10 to 24 hours, thereby causing germination. In this regard, it may be effective to use warmed water containing ozone (which has been known to have a germicidal effect) dissolved therein, since the growth of undesirable microorganisms (e.g., general bacteria, *Escherichia coli*, viruses) in the water during the immersing can be prevented. The ozone-dissolved warmed water may be fed to the brown rice grains by, for example, directly feeding air containing ozone generated by an ozone generator (e.g., Ozone Corporation; Model OZ-2-A100-30) to a hot-water bath in which the brown rice grains are contained, or by circulating ozone-dissolved warmed water from a storage vessel.

The germination level of brown rice is preferably a level at which there can be observed a swelling or protrusion of a germ portion of approximately 1 mm. After germinated, the brown rice grains are dried or processed with heat, or stored at a temperature of not higher than 6°C or freeze-stored.

(2) Determination of PEP-inhibition activity of cereal components

PEP-inhibition activity of a cereal component can be determined by measuring a difference in percent substrate degradation of a synthetic or natural substancewhichcontains

proline residue intramolecularly between a case where a sample containing the inhibitive substance is present and a case where such a sample is absent. In this regard, the synthetic substance may be one having a degradation-indicator attached to the C-terminus, such as Z-Gly-Pro-p-nitroanilide (Z-Gly-Pro-pNA), Z-Gly-Pro-2-naphthylamide, Z-Gly-Pro-4-methylcoumarinamide. The natural substrate may be a natural peptide, such as oxytocin and thyrotropin-releasing hormone stimulating hormone. The PEP may be one derived from *Flavobacterium meningosepticum* (e.g., a product of Funakoshi Corporation) or one isolated and purified from an laboratory animal such as a rat and a mouse.

(3) Isolation/purification of PEP-inhibitive compound

The isolation of the PEP-inhibitive compound of the present invention from the source as described above can be performed as follows.

The source is crushed as is or using a mortar or ball mill. The crushed product is extracted with a solvent (e.g., distilled water, methanol, ethyl acetate, n-hexane). The extract is concentrated to dryness using an evaporator or the like, and then dissolved in an appropriate solvent. The resultant solution is applied on a chromatography column filled with a carrier (e.g., silica gel), and eluted with an appropriate solvent (e.g., an ethyl acetate/n-hexane mixed solution). The active fractions are collected to give a solution containing the crude product of the PEP-inhibitive compound(s) of interest. The solution is subjected to thin-layer chromatography, high-performance liquid chromatography or the like, to thereby isolate the PEP-inhibitive

compound(s).

(4) Structural determination

The chemical structure of the PEP-inhibitive compound obtained in the step (3) can be determined by instrumental analysis utilizing IR spectroscopy, ^{13}C-NMR, ^{1}H-NMR, correlation two-dimensional NMR (e.g., correlation spectroscopy: COSY) and so on in appropriate combination. Once the chemical structure of the PEP-inhibitive compound is determined, the compound may be prepared by a chemical synthetic process.

2. Application of PEP-inhibitive Compound as Food Material

The PEP-inhibitive compound of the present invention can be added to a health food product suited for prevention or improvement of cerebral functional disorders caused by the degradation of cerebral function-associated peptides by PEP which may cause Alzheimer's disease, amnesia and so on. The PEP-inhibitive compound may be added to various types of food products including solid food products, jellied food products and liquid food products. Examples of the solid food product include dough for bread; dough for baked foods such as rice cracker, biscuit and cookie; noodles such as *soba* noodle made from buckwheat and *udon* noodle made from wheat; fish meat products such as *kamaboko* (a Japanese boiled fish paste) and *chikuwa* (a Japanese tubular baked fish paste); butcher's meat products such as ham and sausage; and milk powder. Examples of the jellied food products include fruit jellies and coffee jellies. Examples of the liquid food product include green teas, coffees, black teas, cultured milks and acidophilus beverages. In

particular, a kind of Japanese blend green tea blended with roasted rice grains (known as "*genmai-cha*" in Japan) is preferable. *Genmai-cha* is very popular with Japanese people because of its characteristic nice flavor of roasted rice, and occupies a large part of the daily teas consumed in Japan. *Genmai-cha* can be prepared by drying brown or white rice grains that have been steamed, roasting the dried rice grains and then blending the roasted rice grains with green tea leaves. When germinated brown rice is used as the rice grain, it becomes possible to produce *genmai-cha* having a high dementia-preventive effect by itself.

For the production of the food product, the PEP-inhibitive compound may be added in an isolated/purified form or in the form of an extract or powder of a cereal grain containing the PEP-inhibitive compound (e.g., white rice grain, non-germinated brown rice grain, and germinated brown rice grain). The amount of the PEP-inhibitive compound added to the food product may be within the range from 0.01 to 1 wt%, preferably from 0.1 to 0.5 wt% in an isolated/purified form; from 0.001 to 0.1 wt%, preferably from 0.005 to 0.05 wt% in the form of a crude extract of a cereal grain; or from 1 to 10 wt%, preferably from 2 to 5 wt% in the form of a powder of a cereal grain. However, it is possible to add the PEP-inhibitive compound in an amount outside these ranges, depending on the type or shape of the intended food product, the type of a subject who eats the food product, and so on.

Germinated brown rice contains a remarkably increased amount of the PEP-inhibitive compound(s) compared to white rice or non-germinated brown rice. Accordingly, germinated brown rice may be processed into a cooked rice food product which has been

conventionally produced from white rice, in place of white rice (e.g., a rice cake (*mochi*) and a rice gruel), to provide a daily food effective for preventing Alzheimer's disease or amnesia.

3. Pharmaceutical Formulation Containing PEP-inhibitive Compound as Active Component

The PEP-inhibitive compound of the present invention can be administered to a patient orally or parenterally as an anti-Alzheimer's agent or an anti-amnesiatic agent in the form of a pharmaceutical formulation. The pharmaceutical formulation may contain pharmaceutically acceptable carrier(s) and/or additive(s). Examples of the carrier and additive include water, pharmaceutically acceptable organic solvents, collagens, poly(vinyl alcohol), poly(vinyl pyrrolidone), carboxy vinyl polymers, sodium alginate, water-soluble dextran, sodium carboxymethylstarch, pectin, xanthan gum, gum arabic, casein, gelatin, agar, glycerol, propylene glycol, poly(ethylene glycol), vaseline, paraffin, stearyl alcohol, stearic acid, human serum albumin, mannitol, sorbitol, lactose and pharmaceutically acceptable surfactants. The carrier and additive are suitably selected from these members singly or in combination, depending on the dosage form of the pharmaceutical formulation.

For oral administration, the pharmaceutical formulation may be in a solid form (e.g., tablets, granules, powder and pills), a liquid form (e.g., solution and syrup) or the like. In particular, the granule or powder formulation may be in a unit dosage form such as a capsule. For a liquid dosage form, the pharmaceutical formulation may be in a dried form which is re-dissolved for use. The solid formulation may contain common pharmaceutical additives,

such as binders, excipients, disintegrators, wetting agents and lubricants. The liquid formulation may contain common pharmaceutical additives, such as stabilizing agents, buffering agents, preservatives, flavoring agents, coloring agents and corrigents.

For parenteral administration, the pharmaceutical formulation may be in the form of a formulation for injection, a suppository or the like. In particular, for administration through injection, the pharmaceutical formulation may be in a powder form which is usually contained in a unit-dosage ampoule or multiple- dosage container and re-dissolved in an appropriate carrier (e.g., sterile water free from exothermic substance) for use. These dosage forms may contain common pharmaceutical additives, such as emulsifying agents and suspending agents. The formulation for injection may be administered through intravenous drip, intravenous injection, intramuscular injection, intraperitoneal injection, subcutaneous injection, intracutaneous injection or the like.

The dosage of the pharmaceutical formulation may vary within a wide range depending on the age of a subject, the route of administration, the number of administrations and the like. In general, the dosage is desirably from 1 to 10 mg per an adult for oral administration, or from 10 to 50 mg per an adult for perenteral administration.

4. Assessment of Anti-demential Effect

The anti-demential effect of the PEP-inhibitive compound of the present invention can be determined by commonly employed shuttle test or passive avoidance learning test in rats (Int. Symp.,

On Pharmacology of Learning and Memory, 1981). For example, passive avoidance learning test in rats is performed using a passive avoidance test box composed of a gridded, electrically charged floor and a platform of safety. The rats to be tested are divided into three groups: Group I receiving an injection of the PEP-inhibitive compound of the present invention; Group II (negative control) receiving physiological saline alone; and Group III (positive control) receiving Z-prolylprolynal which is known to have a PEP-inhibition activity. The rats are placed on the platform in the box. When the rats get down from the platform onto the floor, an electric current is passed through the floor until the rats get up onto the platform. Rats that stay on the platform for 20 sec. or longer are recognized as "learned rats", and then taken out from the box. The learned rats are administered with an amnesia-inducing agent, scopolamine hydrobromide, to develop amnesia artificially. The injected rats are placed on the platform in the box again, and the time period of stay on the platform (also referred to as "the staying time period") is measured. If the staying time periods for the rats of Group I (i.e., rats injected with the compound of the present invention) are significantly longer than those for the rats of Group II (negative control), then the compound of the present invention is assumed to exhibit an anti-amnesiatic effect. While if there is no significant difference in the staying time period between the rats of Group I and Group II, then the compound of the present invention is assumed to exhibit no or less anti-amnesiatic effect. When the anti-amnesiatic effect is recognized, the staying time periods for the rats of Group I are compared with those for the rats of Group III

(positive control). If the staying time periods for the rats of Group I are longer than those for the rats of Group III, then the compound of the present invention is assumed to exhibit a higher anti-amnesiatic effect than Z-prolylprolynal. While if shorter than those for the rats of Group III, then the compound of the present invention is assumed to exhibit a lower anti-amnesiatic effect than Z-prolylprolynal.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 illustrates the cleavage sites in cerebral functional peptides by prolylendopeptidase.

Fig. 2 is a graphical illustration of the relation between the PEP-inhibition activity and the extraction time in extracts of germinated brown rice with different solvents.

Fig. 3 is graphical illustration of the relation between the PEP-inhibition activity and the extraction time period in extracts of white rice, brown rice and germinated brown rice with n-hexane.

All publications and patent applications cited herein are incorporated herein by reference in their entirety.

BEST MODE FOR CARRING OUT THE INVENTION

The present invention is illustrated in more detail with reference to the following working examples. However, it should be understood that the invention is not limited to the specific details set forth in the examples.

Example 1

Extraction of compound having PEP-inhibition activity from germinated brown rice

A compound having a PEP-inhibition activity was extracted from germinated brown rice grains with different solvents. Brown rice grains (Brand name: *Nagano Koshihikari*) supplied by a farm (Ueda-shi, Nagano-ken, Japan) were washed with water, and then subjected to germination in a microprocessor-controlled electronic germinator (Made by Takekoshi Seisakusho Co.) for 21 hours. Each of distilled water, methanol, ethyl acetate and n-hexane was added in an amount of 1200 ml per about 450 g of the germinated brown rice grains to perform extraction. A portion of the solvent was sampled every five days after the extraction was started, and the solvent was removed therefrom using an evaporator, thereby yielding an extract which was assumed to contain a compound having a PEP-inhibition activity.

Example 2

Determination of PEP-inhibition activity for the extracts of germinated brown rice with different solvents

The PEP-inhibition activity was determined according to the method of Yoshimoto et al. (T. Yoshimoto et al., Biochim. Biophys. Acta, 569:184-189, 1979). A sample solution was prepared by fully dissolving each of the extracts obtained in Example 1 (0.1 g, each) in a 40% aqueous dioxane solution (2 ml). A substrate solution was prepared by dissolving Z-Gly-Pro-pNA in a 40% aqueous dioxane solution to a final concentration of 2 mM. An enzyme solution was prepared by dissolving PEP derived from *Flavobacterium meningosepticum* (a product of Funakoshi Co.) in 0.05M sodium

phosphate buffer (pH 7.0) to a final concentration of 0.175 U/ml. An enzymatic reaction-termination solution was prepared by dissolving Triton X-100 (10 g) in 1M sodium acetate buffer (95 ml). The enzymatic reactions were performed by using these solutions in the compositions, in the sequence and under the conditions shown in Table 1. For each reaction solution, after the reaction was terminated, the absorbance at 410 nm (OD_{410}) was measured.

Table 1

Reaction systems for determination of PEP-inhibition activity

	Sample	Sample control	Blank	Blank control
0.1M Sodium phosphate buffer	100 μl	100 μl	100 μl	100 μl
Sample solution	125 μl	125 μl	0 μl	0 μl
Substrate solution	125 μl	125 μl	125 μl	125 μl
Pre-incubation (30°C, 5min)	Yes	Yes	Yes	Yes
Reaction termination solution	0 μl	2000 μl	0 μl	2000 μl
Incubation (30°C, 10min)	Yes	Yes	Yes	Yes
Reaction termination solution	2000 μl	0 μl	2000 μl	0 μl
OD_{410}	S	S′	B	B′

The measured absorbance values B, B′, S and S′ were substituted in the following equation to yield a PEP-inhibition activity for each extract.

$$\text{PEP-inhibition activity (\%)} = [(B-B')-(S-S')]\div(B-B')\times 100$$

As shown in Fig. 2, the n-hexane extract showed a high PEP-inhibition activity of higher than 50% 10 days after the extraction was started. In the extracts with other solvents showed PEP-inhibition activities of lower than 50% even 20 days after the extraction was started, which was about the same level as that

determined on day 5. These results indicated that the n-hexane extract contained a particular PEP-inhibitive compound(s) in a larger amount than the extracts with other solvents, or that a particular compound(s) having a high PEP-inhibition activity was only contained in the n-hexane extract. In view of the fact that high PEP-inhibition activity was observed in an organic solvent extract (i.e., n-hexane) rather than a distilled water extract, it was found that a particular lipid-soluble substance was involved in the inhibition of PEP.

Example 3

Isolation/purification of PEP-inhibitive components from n-hexane extract

The n-hexane extract (200 mg) obtained in Example 1 was dissolved in a small volume of n-hexane. The resulting solution was applied on the top of a glass column (500 mm × 50 mm) that had been filled about 60% full of silica gel 60 (70-230 mesh), and then eluted with portions of an ethyl acetate/n-hexane mixed solution (100 ml each) with gradually increasing the concentration from 10% to 70% by 10%. The combined effluents were fractionated into fractions C-1 to C-6 on the basis of the thin-layer chromatographic isolation (Table 2). These fractions were separately concentrated and dissolved in n-hexane for storage. Among these fractions, C-2 and C-3 were subjected to structural determination as set forth below.

Table 2

Rf values for spots of the individual fractions

on thin-layer chromatography

Fraction #	Rf value
C-1	0.90, 0.84, 0.80, 0.70, 0.66
C-2	0.66
C-3	0.38
C-4	0.38, 0.36, 0.30, 0.26
C-5	0.36, 0.26
C-6	0.20, 0.16, 0.10, 0.04, 0.02

Example 4

Structural determination of PEP-inhibitive compounds

(1) Chemical structure of Compound C-2

According to IR spectrometry of Compound C-2, a characteristic absorption band of carbonyl radical was observed at 1740 cm^{-1}. According to ^{13}C-NMR analysis of Compound C-2, 35 carbon signals shown in Table 3 were observed.

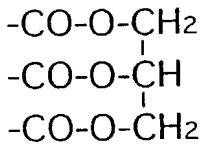
Table 3

NMR spectra of compound C-2 ($CDCl_3$)

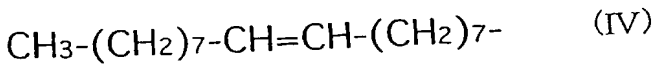
	^{13}C-NMR	DEPT	^{1}H-NMR	Number of H atom(s)
1	173.935	C		
2	173.901	C		
3	173.483	C		
4	130.898	CH	5.34	1H
5	130.692	CHX2	5.34	2H
6	130.655	CH	5.34	1H
7	130.388	CH	5.34	1H
8	130.361	CH	5.34	1H
9	128.776	CH	5.34	1H
10	128.760	CH	5.34	1H
11	128.588	CH	5.34	1H
12	128.579	CH	5.34	1H
13	69.592	CH	5.26	1H
14	62.783	CH2X2	4.28 4.13	4H
15	34.712	CH2X3	2.37	6H
16-28	32.587-29.772	CH2X26	1.3	52H
29	27.908	CH2X2	2.02	4H
30	27.883	CH2X2	2.02	4H
31	27.858	CH2X2	2.02	4H

32	26.235	CH2	2.7	2H
33	25.529	CH2X3	1.6	6H
34	23.358	CH2X2	1.3	4H
35	14.774	CH3X3	0.9	9H

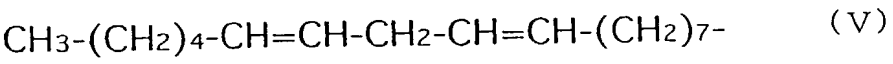
The correlation signals obtained from HMBC, COSY were also analyzed. From these results, it was revealed that Compound C-2 was a triglyceride having the partial structures of formulae (III) to (V).



(III)



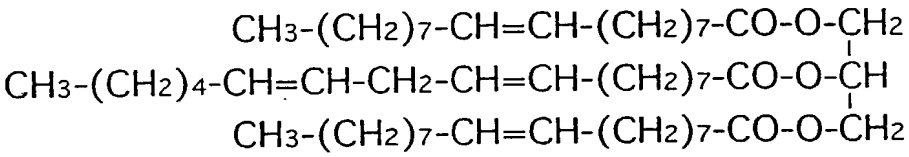
(IV)



(V)

From the fact that Compound C-2 showed no optical activity, it was also revealed that fatty acids bonded to positions 1 and 3 of the triglyceride were identical to each other. The integral ratio in ^{1}H-NMR spectra and the analytical date from the two-dimensional NMR spectroscopy revealed that an oleic acid was bonded to each of the fatty acids at positions 1 and 3 of the triglyceride and linoleic acid was bonded to the fatty acid at position 2 of the triglyceride. From these results, it was found that Compound C-2 was 1,3-dioleoyl-2-linoleoyl glycerol of formula (I).

(I)



(2) Chemical structure of Compound C-3

According to IR spectrometry of Compound C-3, a characteristic absorption band of ketone radical was observed at 1700 cm^{-1}. According to ^{13}C-NMR spectroscopic analysis of Compound C-3, 28 carbon signals shown in Table 4 were observed.

Table 4

NMR spectra of compound C-3 ($CDCl_3$)

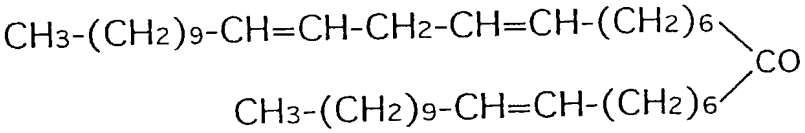
	^{13}C-NMR	DEPT	^{1}H-NMR	Number of H atom(s)
1	180.108	C		
2	130.242	CH	5.34	1H
3	130.049	CHX2	5.34	2H
4	129.751	CH	5.34	1H
5	128.108	CH	5.34	1H
6	127.939	CH	5.34	1H
7	34.074	CH2X2	2.34	4H
8	31.934	CH2	1.28	2H
9	31.560	CH2	1.28	2H
10	29.796	CH2X2	1.28	4H
11	29.706	CH2X2	1.28	4H
12	29.613	CH2X2	1.28	4H
13	29.549	CH2X2	1.28	4H
14	29.376	CH2X2	1.28	4H
15	29.350	CH2X2	1.28	4H
16	29.343	CH2X2	1.28	4H
17	29.164	CH2X2	1.28	4H
18	29.093	CH2X2	1.28	4H
19	29.062	CH2X2	1.28	4H
20	27.247	CH2	2.02	2H
21	27.211	CH2	2.02	2H
22	27.184	CH2	2.02	2H
23	25.663	CH2	2.77	2H
24	24.695	CH2X2	1.63	4H
25	22.705	CH2	1.28	2H
26	22.596	CH2	1.28	2H
27	14.115	CH3	0.88	3H
28	14.072	CH3	0.88	3H

The signals of ^{13}C-NMR spectra were numbered from the lowest magnetic field intensity to the highest magnetic field intensity (i.e., No.1-28). The correlation signals obtained by HMBC , COSY were analyzed. As a result, it was presumed that Compound C-3 had the partial structures of formulae (VI) and (VII).

$$CH_3\text{-}(CH_2)_9\text{-}CH{=}CH\text{-}CH_2\text{-}CH{=}CH\text{-}(CH_2)_6\text{-} \quad \text{(VI)}$$

$$CH_3\text{-}(CH_2)_9\text{-}CH{=}CH\text{-}(CH_2)_6\text{-} \quad \text{(VII)}$$

From these results, it was found that Compound C-3 was 7-octadecenyl-7,10-henicosadienyl ketone of formula (II).



(II)

Example 5

Comparison in PEP-inhibition activity of n-hexane extract among white rice, non-germinated brown rice and germinated brown rice

Since the n-hexane extract of germinated brown rice showed a high PEP-inhibition activity, n-hexane extracts of white rice and non-germinated brown rice were also prepared, and PEP-inhibition activity was compared among these three kinds of n-hexane extracts.

White rice grains and non-germinated brown rice grains that

had been soaked in distilled water for 21 hours were separately extracted with n-hexane in the same manner as for germinated brown rice grains. For the extraction, 1200 ml of the extraction solvent (i.e., n-hexane) was used per 450 g of rice grains. The PEP-inhibition activity of each of the extracts was determined every five days after the extraction was started. In this experiment, a sample solution was prepared by dissolving the extract (0.1 g) in a 40% aqueous dioxane solution (2 ml). The results of the PEP-inhibition activities of the individual n-hexane extracts are shown in Fig. 3.

The comparison of the PEP-inhibition activity was performed for the extracts sampled every five days after day 10 of the extraction. There was observed no significant difference in PEP-inhibition activity of the extract between white rice and non-germinated brown rice. However, the extract of germinated brown rice showed a higher PEP-inhibition activity compared to the extracts of white rice and non-germinated brown rice. From these results, it is presumed that the content of the particular PEP-inhibitive compound(s) increases in brown rice during the germination process, or that any additional PEP-inhibitive compound(s) is produced in brown rice during the germination process. With respect to white rice and non-germinated brown rice, there is observed no or little difference in PEP-inhibition activity of the extract between them. However, the amount of PEP-inhibitive compound(s) extracted from white rice is about one-half those which were extracted from non-germinated brown rice and germinated brown rice. Consequently, it is found that a larger amount of the PEP-inhibitive compound(s) is contained in brown rice

compared with white rice.

INDUSTRIAL APPLICABILITY OF THE INVENTION

According to the present invention, as described above, there can be provided a PEP inhibitor comprising an extract from a cereal grain as an active component; a method for preparing the PEP inhibitor; a substance having a PEP-inhibition activity, which is extracted and purified from a cereal grain; a method for preparing the substance; a PEP inhibitor comprising the substance; a food product for preventing or improving cerebral dysfunction, which comprises the substance; a germinated brown rice for preventing or improving cerebral dysfunction, which has a PEP-inhibition activity; and a food product for preventing or improving cerebral dysfunction, which comprises the germinated brown rice. The present invention is useful for preventing or reducing the symptoms of patients having a cerebral functional disorder (e.g., dementia and amnesia).

All publications and patent applications cited herein are incorporated herein by reference in their entirety.

WHAT IS CLAIMED IS:

1. A prolylendopeptidase-inhibitive agent comprising an extract of a cereal grain as an active component.

2. The prolylendopeptidase-inhibitive agent according to claim 1, wherein the cereal grain is a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame.

3. The prolylendopeptidase-inhibitive agent according to claim 2, wherein the grain of rice plant is in a germinated form.

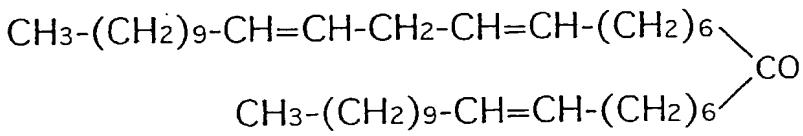
4. A method for preparing a prolylendopeptidase-inhibitive agent comprising extracting a cereal grain with water and/or an organic solvent.

5. A method according to claim 4, wherein the cereal grain is a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame.

6. The method according to claim 5, wherein the grain of rice plant is in a germinated form.

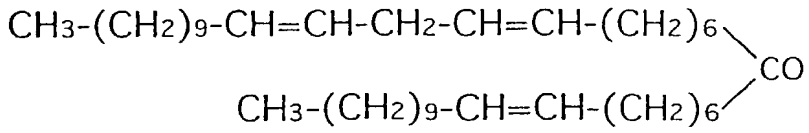
7. The method according to claim 4, wherein the organic solvent is hexane.

8. A compound of formula (II).



(II)

9. A method for preparing a compound of formula (II), comprising extracting a cereal grain and then isolating the compound from the extract.



(II)

10. The method according to claim 9, wherein the cereal grain is a grain or seed of at least one member selected from the group consisting of rice plant, wheat, corn, soybean, milo, buckwheat, foxtail millet, barnyard grass, proso and sesame.

11. The method according to claim 10, wherein the grain of rice plant is in the form of germinated brown rice.

12. A prolylendopeptidase-inhibitive agent comprising a compound as claimed in claim 8 as an active component.

13. A food product for preventing and/or improving cerebral dysfunctions, the food product comprising a compound as claimed in claim 8 as an active component.

14. A germinated brown rice for preventing and/or improving cerebral dysfunctions, the germinated brown rice having a

prolylendopeptidase-inhibition activity.

15. A food product for preventing and/or improving cerebral dysfunctions, the food product comprising a germinated brown rice having a prolylendopeptidase-inhibition activity.

ABSTRACT

Disclosed is a prolylendopeptidase-inhibitive agent. More specifically, there are provided a prolylendopeptidase-inhibitor comprising an extract of a cereal grain as an active component; a method for preparing the prolylendopeptidase-inhibitor; a compound having a prolylendopeptidase-inhibition activity, which is extracted and isolated from a cereal grain; a method for preparing the compound; a prolylendopeptidase-inhibitive agent comprising the compound; a food product for preventing and/or improving cerebral dysfunctions, which comprises the compound; a germinated brown rice for preventing and/or improving cerebral dysfunctions, which has a prolylendopeptidase-inhibition activity; and a food product for preventing and/or improving cerebral dysfunctions, which comprises the germinated brown rice.

FIG.1

Substance P	Arg-Pro-Lys-Pro↓Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
Neurotensin	Pry-Leu-Tyr-Gln-Asn-Lys-Pro-Arg-Arg-Pro↓Tyr-Ile-Leu
Vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys-Pro↓Arg-Gly-NH_2
Oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro↓Leu-Gly-NH_2

FIG.2

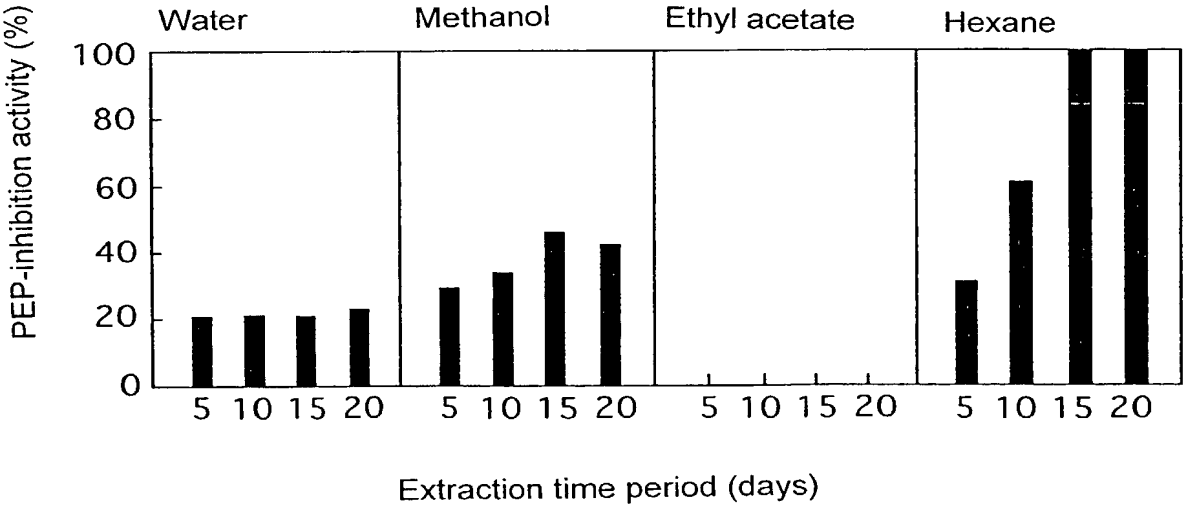
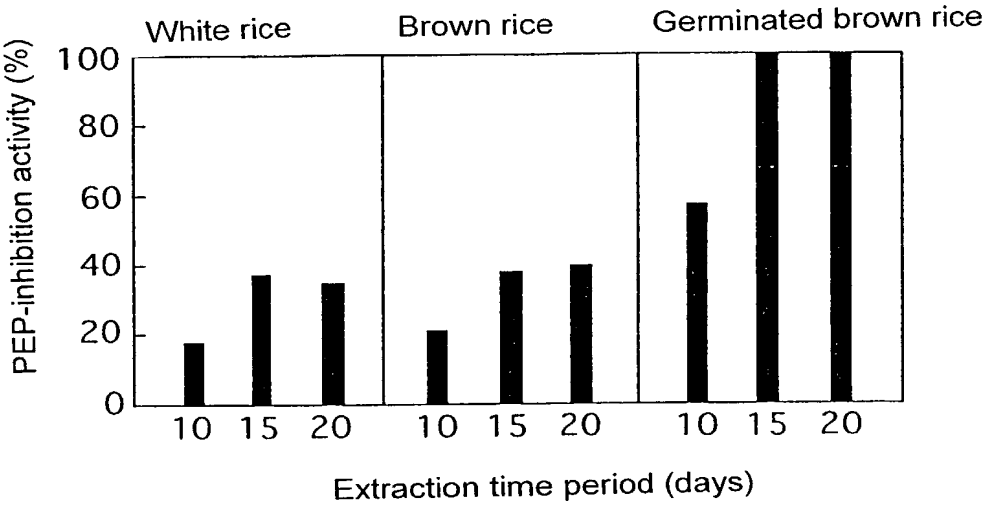


FIG.3


White rice
Brown rice
Germinated brown rice
PEP-inhibition activity (%)
100
80
60
40
20
0
10 15 20
10 15 20
10 15 20
Extraction time period (days)

Attorneys Docket No.: 216120US0PCT

DECLARATION, POWER OF ATTORNEY AND PETITION

I (We), the undersigned inventor(s), hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I (We) believe that I am (we are) the original, first, and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

PROLYENDOPEPTIDASE INHIBITORS

the specification of which

☐ is attached hereto.

■ was filed on November 19, 2001 as Application Serial No. 09/926,566 and amended on _____.

■ was filed as PCT international application Number PCT/JP00/03135 on May 16, 2000, and was amended under PCT Article 19 on _____ (if applicable).

I (We) hereby state that I (We) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; that I (We) do not know and do not believe that this invention was ever known or used before my invention or discovery thereof, or patented or described in any printed publication in any country before my invention or discovery thereof, or more than one year prior to this application, or in public use or on sale in the United States for more than one year prior to this application; that this invention or discovery has not been patented or made the subject of an inventor s certificate in any country foreign to the United States on an application filed by me or my legal representatives or assigns more than twelve months before this application.

I (We) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

I (We) hereby claim foreign priority benefits under Section 119(a)-(d) of Title 35 United States Code, of any foreign application(s) for patent or inventor s certificate listed below and have also identified below any foreign application for patent or inventor s certificate having a filing date before that of the application on which priority is claimed:

Application No.	Country	Filing date	Priority claimed
138791/1999	Japan	May 19, 1999	■ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

I hereby claim the benefit under Section 119(e) of Title 35 United States Code, of any United States application(s) listed below.

(Application Number)	(Filing Date)
(Application Number)	(Filing Date)

I (We) hereby claim the benefit under Section 120 of Title 35 United States Code, of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Section 112 of Title 35 United States Code, I (We) acknowledge the duty to disclose material information as defined in Section 1.56(a) of Title 37 Code of Federal Regulations, which occurred between the filing date of the prior application and national or PCT international filing date of this application:

Application Serial No.	Filing Date	Status (pending, patented, abandoned)

(30)

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I (We) declare further that all statements made herein of my (our) knowledge are true and that all statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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